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ON CALCIUM METABOLISM

by

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ON CALCIUM METABOLISM

Certain lines of enquiry in biological chemistry have already yielded results such that a coherent and plausible account can be given of the phenomena involved and their relationships, though doubtless in no case can the final story be told. At the present time, however, our knowledge of calcium metabolism is fragmentary - we are still far from being able to correlate the various experimental results and employ them in the weaving of a single harmonious hypothesis; the gaps are too great, and much of what passes for knowledge is uncertain. As a result of this almost chaotic array of contradictory statements deranged calcium metabolism has been suggested as the etiological factor in the most diverse conditions, and this has in turn led to much misdirected and futile therapeutic endeavour. The object of this thesis is to put forward the results which have been obtained by the author in his investigations into several problems of calcium metabolism, to correlate these with the results obtained by previous workers, and to present a short critical review of the more important and suggestive papers on this subject as it is understood to-day.

That/

That calcium plays an important part in almost all the bodily functions has long been an acknowledged fact, and amongst the older physicians it was strongly emphasised by Parry⁽¹⁾, in whose writings reference is made to a possible relationship between absence of calcium and the presence of malnutrition. The application of chemical methods of analysis to the study of calcium metabolism seems to have been first made by Schmidt⁽²⁾, but it was not until the advent of such methods of estimation as those of Lyman, McCruden, Howland and Kramer, de Waard, and especially of Kramer and Tisdall that much real progress became possible.

Doubtless, the relationships between calcium and the other inorganic substances present in living tissues are very complex, and the complexity is not diminished by the presence of organic ampholytes. Moreover, apart even from these obstacles to a clear understanding of the metabolism of calcium, there appear to be other less obvious factors to which at present we possess no clue. In this connection one may mention the apparently well established fact that calcium chloride, fed by the mouth, is absorbed to a much greater extent than is calcium lactate.⁽³⁾ This assertion rests on no isolated observation; it has been made time and again by reliable workers who have adduced what appears to be ample evidence. Yet in either case, one would imagine, the effect of the gastric/

gastric juice would be the production of free calcium ions in the presence of a large excess of chlorions. In the intestine, where, presumably, absorption takes place to the greatest extent (if not exclusively), it is true that the reaction may be alkaline, but the anion present in greatest concentration is still the chlorion. Why, then, should calcium chloride be absorbed so much more easily than the lactate?

Calcium Requirements for Growth and Maintenance.

It has long been recognised in a general way that the inorganic components of the diet play a role that cannot be neglected; nevertheless, until quite recently the mineral nutrients have largely been considered in toto only, their content being expressed as the per cent. of 'ash' yielded by a given food, ration, tissue, or organ. A supply of calcium is obviously indispensable to a growing and bone-producing, or milk-producing animal, but it was not until 1918 that Osborne and Mendel⁽⁵⁾ demonstrated the growth-retarding influence of a diet adequate in every respect except as regards its calcium content, and the immediate recommencement of growth and improvement in nutrition on the same diet when the daily intake of calcium was augmented by the addition of calcium salts, thus proving that a certain minimum allowance of calcium was necessary for growth and maintenance.

Sherman and his co-workers^(6,7) studied fully the/

the question of calcium maintenance allowance in human subjects receiving a calcium ration slightly less than the minimal requirement, and they found that the body was unable to adjust itself to a metabolism of less than 0.29-0.49 grams calcium per 70 kilos. per day. From these data, and from a study of the available figures in the literature which appeared to be reliable and to have been obtained by methods comparable to his own, Sherman concludes that it seems probable that the average 0.45 grams calcium per 70 kilos. per day does approximately represent the minimum maintenance requirement for normal human nutrition. He suggests, further, that an adequate supply should contain 1 gram calcium (1.4 gm. CaO) per 100 grams protein, and, from a comparison of dietary studies and laboratory evidence of nutritive requirements, is of opinion that a considerable proportion of dietaries are deficient in quantity of calcium.

In the growing child the amount per kilo. body weight is naturally greater, and Herbst⁽⁸⁾ states that the normal child stores about 0.3 grams of calcium per day. Even in a well balanced diet, of course, calcium must be present in amounts much greater than these to allow for the low efficiency of absorption - 1.0 to 1.5 grams of calcium seems to be the average daily dietary requirement.⁽⁹⁾

The/

The Absorption of Calcium.

Calcium salts, whether soluble or insoluble, appear to be absorbed with considerable difficulty. Even under the most favourable conditions, the greater part of the ingested calcium is lost in the faeces, having apparently escaped absorption. Nevertheless, a certain amount is assimilated.

Calcium is present in the diet both in organic combination - e.g. as calcium proteinate, soaps, etc. - and in inorganic combination as chloride, phosphate, bicarbonate, etc., and many efforts have been made to determine the relative availability of these forms of combination. There is a certain amount of evidence in support of the view that calcium as proteinate is more readily absorbed than certain of the simpler calcium salts. Thus Givens^(10,11) found that ingestion of milk was followed by a greater excretion of calcium in the urine than was ingestion of an equivalent amount of calcium lactate. Further, Sherman and Hawley⁽¹²⁾ and also McClugage and Mendel⁽¹³⁾ showed that in children vegetables were inferior to milk as a source of calcium, though Rose⁽¹⁴⁾ claims that in adults carrots form as good a source of calcium as does milk.

Since, however, many factors other than chemical combination have been found to influence the absorption of calcium, we are on surer ground in comparing the absorption of different simple calcium salts than in comparing the availability of inorganic and/

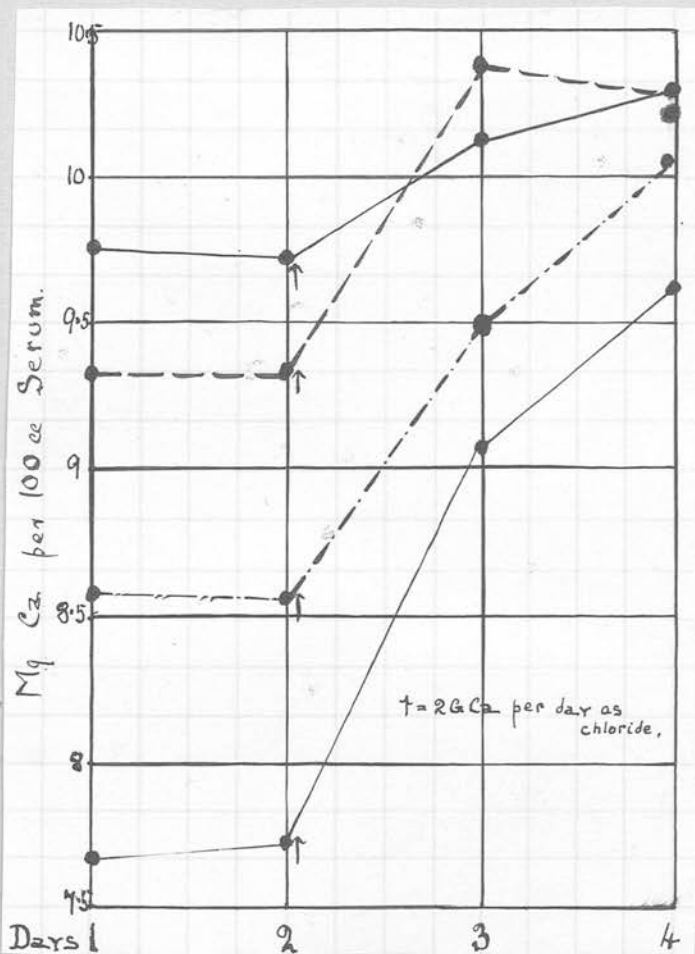
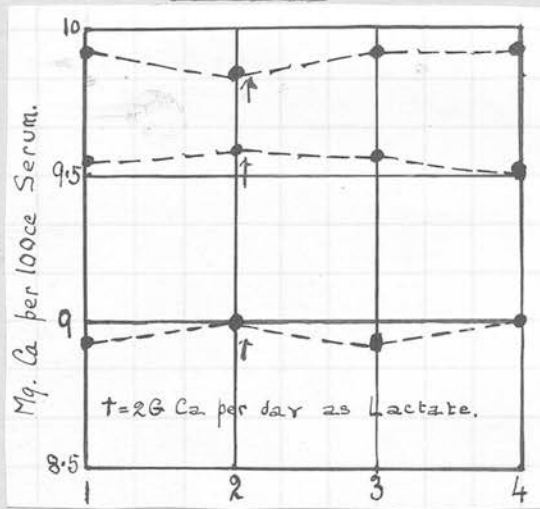
and organic salts. It has already been mentioned that calcium chloride appears to be absorbed with much greater ease than calcium lactate, though even this salt undergoes some absorption. Mason⁽¹⁵⁾ and Denis and Minot⁽³⁾ report that calcium lactate failed to produce any increase in the calcium content of normal human serum; Kramer and Howland⁽¹⁶⁾ obtained similar results in rats, and Clark⁽¹⁷⁾ in rabbits. Howland and Marriott⁽¹⁸⁾, too, found that calcium chloride was more efficient than the lactate in the relief of tetany.

That some absorption of the lactate does take place, however, is shown by the rise in the serum calcium when calcium lactate is fed to animals having a low initial value⁽³⁾, and by the absence of active tetany in parathyroidectomised dogs fed on a meat diet with addition of calcium lactate⁽¹⁹⁾. The greater ease with which calcium chloride is absorbed is shown by the increase in the serum calcium following its ingestion by normal animals.⁽¹⁵⁾ Similarly Matz⁽²⁰⁾ found that in normal and tuberculous subjects ingestion of inorganic calcium salts resulted in a rise in the serum calcium, the rise with calcium chloride being greater and more prolonged than with the lactate.

Experiments have been carried out by the author on adult human subjects to test the relative availability for absorption of calcium chloride and calcium lactate. These salts were given in solution, by the mouth/

mouth and on an empty stomach, in doses containing equivalent amounts of calcium, and the absorption judged by the subsequent serum calcium values. The results show that while the administration of calcium chloride was invariably followed by an increase in the serum calcium, the lactate produced no such rise.

Graph I.



(21)
Hjort ,on the other hand, claims that calcium, whether given as the chloride, lactate, or glycerophosphate, definitely increases the serum level provided it is administered in amounts greater than 0.2727 grams of CaO per kilo. body weight, while with relatively insoluble calcium salts such as the carbonate the results are inconstant . Steenbock, Hart, Sell, and Jones (22) go even further, for they found no difference in the availability of calcium lactate, carbonate, phosphate, silicate, or sulphate when these were fed to young rats in liberal amounts. These authors claim that solubility in hydrochloric acid is an essential factor in determining the availability of calcium salts, for the contents of the small intestine remain acid for a considerable time, and often throughout its entire length (23, 24) . This idea, which explains the absorption of salts insoluble in water or alkali receives some support from other workers. Thus Zucker and Matzner (25) found that in cases of active untreated rickets (in rats) the faeces were alkaline, a condition unfavourable to calcium absorption; after cod-liver oil administration and simultaneously with clinical improvement, the intestinal contents became acid. Moreover, it has been shown (26) that in rickets the acid secreting function of the stomach is impaired, but improves in convalescence, while Lamb and Evard (27) have found increased calcium retention in the pig following ingestion of lactic and acetic acids.

Bergheim⁽²⁸⁾ has found an interesting relationship between calcium absorption and the sugar content of the diet. The absorption of calcium is appreciably increased when glucose, fructose, maltose or starch is added to make fifty per cent. of the total diet, and is pronouncedly increased by addition of twenty-five per cent. of lactose. He attributes the effect to an increased formation of lactic acid with consequently an increased acidity of the intestinal contents - a condition which appears to favour calcium absorption. Lactose, however, did not prevent the onset of rickets when added to a diet with a low vitamin D content and a high Ca/P ratio.

The assimilation of calcium is affected by many dietary factors. Very important is the balance of the mineral constituents of the diet. While a large excess of sodium chloride, or, in general of chlorion, appears to be a distinct aid to calcium absorption⁽²⁹⁾, an excess of potassium^(30,31), magnesium⁽³²⁾, or phosphate, is definitely detrimental. So great is the influence of these substances, especially in the growing animal, that a diet in which the phosphate is greatly in excess of the calcium, though both are far above the actual requirements, leads, almost invariably, to deficient bone formation and rickets.⁽³⁷⁾

This influence of a large excess of phosphate is perhaps no more than would be expected when one considers the insolubility of calcium phosphate. That potassium/

potassium should have a similar effect is not so obvious, but has been explained by Seemann⁽³⁰⁾ who states that the ingestion and absorption of excess potassium results in the excretion of the excess along with chlorine ions, that the resulting chlorine depletion brings about a deficient secretion of hydrochloric acid in the gastric juice, and that with this abnormally low concentration of chloride in the digestive fluids, calcium cannot be properly absorbed. Zander⁽³¹⁾, in supporting this hypothesis, remarks that whereas in the case of healthy infants the mother's milk contains sodium and potassium in the ratio of 2:1, in the case of rachitic children the ratio may be 1:2. Excess of magnesium in the diet, besides interfering with the absorption of calcium, appears to prevent its proper utilisation, causing an increased urinary excretion, an effect similar to that of potassium or sodium.⁽³⁸⁾

Many workers have observed that the absorption of calcium is greatly aided by the addition of fat to the diet^(34,39). It is difficult to picture the mechanism of such a fat action - the existence of which has been denied by others^(40,41,42,43) - and indeed, it has been stated that calcium soaps are not absorbed^(44,45), which would explain the observation of Givens⁽⁴⁶⁾ that poor utilisation of fats may prevent the storage of calcium even when the supply of that element is abundant. One would imagine that the presence of fatty acids in amounts greater than usual would/

would aid the formation of insoluble calcium soaps and so depress the absorption of calcium in much the same way as excess of phosphate is known to do. Moreover, such a relatively simple - and soluble - salt of calcium as the lactate is, as has already been mentioned, known to be absorbed with difficulty. These considerations, supported by the observation that only some fats, at any rate, were efficacious in improving calcium absorption^(43,41), naturally led to attention being turned to the vitamin content of fat as a possible explanation. McCollum and his co-workers⁽⁴⁷⁾ found, however, that cod-liver oil was equally effective whether administered fresh or after oxidation for twelve hours whereby its content of vitamin A had been entirely destroyed. Further, butter fat, though rich in vitamin A, was one of those fats which did not greatly assist the absorption of calcium^(41,43). It was also found by Husband, Godden and Richards⁽³⁴⁾ that, except in extreme cases, olive oil aided calcium absorption to the same extent as did cod-liver oil, and that linseed oil was equally effective. It was obvious, therefore, that the effects were not due to vitamin A, and they were apparently not due to the fat itself, it was necessary to postulate an anti-rachitic factor distinct from vitamin A. More recent work, developing from the effect of ultra-violet radiation, first of the animal itself, later of the whole diet, and then of otherwise inactive fats, has given great support to this idea^(48,49). It has led to the view that/

that fat contains a special anti-rachitic factor - vitamin D - which is more resistant to oxidation than is vitamin A (and so was not destroyed in McCollom's experiments with cod-liver oil), is not necessarily present in large amount in fats rich in vitamin A (it is present in butter fat only in low concentration), and can be produced in otherwise inactive fats such as olive oil by exposure to ultra-violet light. Vitamin D appears to be a derivative of some complex alcohol closely related to cholesterol⁽⁵⁰⁾. In the work of Husband, Godden and Richards no mention is made of possible exposure of the now recognised inert oils used by them to ultra-violet light, and on this fact may rest the explanation of these authors' results which are directly opposed to those of most other workers.

The recent advances in our knowledge of the properties and sources of the anti-rachitic factor, vitamin D, throw much light on the whole question of calcium absorption. The presence, even in minute quantities, of this active substance in the dietary greatly aids the absorption of calcium, and conversely, in its absence, calcium absorption does not proceed readily. Thus, several observers have noted the absence, from fresh green vegetables, of any substance endowed with antirachitic properties, and this fact will adequately explain the low calcium absorption which occurs when these foods, themselves rich/

rich in calcium, form the bulk of the diet⁽⁵¹⁾. On the other hand, milk, while containing a large amount of calcium, is also rich in vitamin D, and hence calcium absorption is increased when milk is fed. Furthermore, Chick^(52,53) has shown that the vitamin D content of milk varies according to whether the milk has been obtained from pasture fed (insolated) or stall-fed animals, the content being higher the more complete the insolation, so that samples of milk even although containing equal amounts of calcium will vary in their effect on calcium absorption according to the conditions to which the milking animal has been subjected. In the opinion of Bergheim⁽⁵⁴⁾ it acts by increasing not the absorption but the retention of calcium, for he found that in rachitic animals calcium was absorbed from the small intestine only to be re-excreted into the large. He suggests that it may act by promoting the breakdown of organic phosphates, thus leading to the increased calcium deposition and lessened gut excretion.

The Excretion of Calcium.

It is usually supposed that calcium is excreted both by the kidney and through the epithelium of the large intestine^(34,55), the greater part going by the latter route. This view has been supported by Van Noorden⁽⁵⁶⁾ and by Grosser⁽⁵⁷⁾, who, avoiding difficulties of absorption by intravenous injection of calcium salts, demonstrated the presence of calcium in the bowels.⁽⁵⁵⁾ Cushny states that, generally speaking, an ion which forms a soluble salt with calcium causes the appearance of that element in the urine, while an insoluble combination tends to appear in the faeces, and that the increased faecal and decreased urinary excretion of calcium caused by excess of phosphate in the diet is not wholly due to decreased calcium absorption.

⁽⁵⁸⁾
Telfer has recently published an account of calcium balance experiments on infants, and concludes from his very suggestive results that the kidney provides the sole excretory route for calcium, stating further that he can find no evidence for excretion into the gut. The method of investigating such a problem by means of balance experiments, where comparatively/

comparatively large quantities of calcium are ingested and only a very small amount is actually absorbed, is highly unsatisfactory, and the experimental error which is possible even with the most careful analysis is necessarily so great as to render the results obtained highly untrustworthy.

In order to obtain direct evidence as to whether the epithelium of the large intestine furnished a means of excretion for calcium, the author carried out the following experiments on isolated loops of colon, thus avoiding difficulties in the interpretation of the results due to the possible presence of unabsorbed ingested calcium.

The cat was the experimental animal employed, and the following technique was adopted.

The cat was anaesthetised by paraldehyde and ether, and placed on artificial respiration throughout the experimental period. Cannulae were inserted into the carotid artery and the external jugular vein. The abdomen having been opened, the small intestine was cut across between two ligatures close to the ileo-caecal valve. The large intestine was brought out and the pelvic colon cut across at its junction with the rectum, all bleeding points being ligated. A large bore cannula was inserted into the tip of the caecum. The isolated loop of large intestine was thoroughly washed out with warm distilled water through the cannula, the removal of solid and semi-solid material and mucous being aided by gently squeezing the bowel with/

with the fingers. Washing was continued until a clear sample was obtained, and this was reserved for analysis. After every washing a final clear sample was collected separately and analysed. Only those experiments were considered in which this sample contained merely traces of calcium and so indicated that the washing had been complete.

After this preliminary washing the isolated loop of intestine was replaced within the abdomen, with the two ends slightly protruding, so that any contents would not escape. The washing was repeated after three and after six hours. In a number of experiments a cannula was inserted into the bladder and samples of urine collected (with washing) at the same time as the intestine was irrigated. Table I, giving the results of experiments of this type, shows that calcium was excreted into the gut and that this excretion occurred at a fairly constant rate. Further, it was usually greater than the urinary excretion for the same period.

Table I /

Table I.

The excretion of calcium by the large intestine and by the kidney in the cat.

<u>Expt.</u>	Wt. of cat in gms.		Mg. Ca in intestinal washings.	Mg. Ca in urine.	Mg. Ca per 100 c.c. serum.		
					At start of Expt.	10 mins. after injection.	At close of Expt.
1	2900	1st 3 hrs.	0.68	0.21	-	-	-
		2nd 3 hrs.	0.66	0.14	-	-	-
2	2540	1st 3 hrs.	0.88	0.17	-	-	-
		2nd 3 hrs.	0.90	0.19	-	-	-
3	3010	1st 3 hrs.	0.33	0.22	10.2	22.0	15.0
		5 c.c. 10% CaCl_2 inj. intravenously	-	-	-	-	-
		2nd 3 hrs.	4.27	0.16	-	-	-
4	2700	1st 3 hrs.	0.68	0.16	-	-	-
		5 c.c. 10% CaCl_2 inj. intravenously	-	-	-	-	-
		2nd 3 hrs.	14.0	0.15	-	-	-
5	2890	1st 3 hrs.	0.30	0.21	9.0	21.0	10.0
		5 c.c. 10% CaCl_2 inj. intravenously	-	-	-	-	-
		2nd 3 hrs.	9.60	0.23	-	-	-

It/

It remained possible that the calcium found in the large intestine did not constitute a true excretion, but had merely been secreted along with the mucous. To test this point, I allowed a preliminary control period of three hours, then injected calcium salts intravenously, and again washed out the gut after a further three hours. After the injection of calcium salt the excretion of calcium into the intestine was enormously increased although there was no corresponding increase in the mucous secretion. The urine did not show any comparable increase in calcium content (Table I). It seems, then, that not only is calcium excreted by way of the large intestine, but that this is the main excretory route.

Nelson and Givens⁽¹⁰⁾ found that about 0.41-0.49 grams calcium were excreted in the urine by adult human subjects of both sexes, on an average diet, while Sherman⁽⁷⁾ found 0.07-0.15 grams calcium to be excreted daily in the urine by subjects receiving a minimal calcium allowance.

As might be expected those substances which influence the absorption of calcium also directly modify the amount excreted in the urine, since a comparison of the figures given by Sherman and by Nelson and Givens indicates that the urinary calcium is to some extent dependent on the amount of calcium absorbed.

The Calcium Content of Blood.

Very wide variations are found in the figures given in the literature for the calcium content of whole blood, serum, plasma, and corpuscles. To no small extent this is due to the different methods which have been used for its estimation. Few of the older methods were trustworthy, and some even of the more recent ones must be regarded with some suspicion.

The methods available for the estimation of calcium in blood are of all types, colorimetric, nephelometric, gravimetric, volumetric, and even, in one case, the simple counting of particles of precipitated calcium oxalate. In the experience of the author, the method of Kramer and Tisdall⁽⁶⁰⁾ has hitherto proved the most reliable, and to be capable of yielding results with a maximum error of five per cent. and an average error of only two per cent. As applied to blood serum, the method consists in precipitating the calcium directly from 1-2 c.c. of serum by means of ammonium oxalate, collecting and washing the precipitate by centrifuging, and finally titrating, in presence of sulphuric acid, with potassium permanganate. The recently published method of Trevan and Bainbridge⁽⁶¹⁾ appears to yield results which are at least equally satisfactory. In this method, the calcium oxalate, precipitated as described by Kramer and Tisdall, is converted to the carbonate by heating, the /

the calcium carbonate is dissolved in standard acid, and the excess acid titrated with alkali by means of a micrometer burette.

Marriott and Howland⁽⁶²⁾, while advocating that for purposes of comparison, estimations should be carried out on serum rather than on whole blood, have stated that there is practically no calcium present in the corpuscles, and they base this statement on their finding that whole blood contains approximately half as much calcium as does serum.

Kramer and Tisdall⁽⁶³⁾, using their own method of analysis, examined the calcium content of whole blood and also of serum in the same samples, along with hematocrit readings. From these data they conclude that the corpuscles contain no calcium. Falta⁽⁶⁴⁾ and Richter Quittner⁽⁶⁵⁾ failed to find calcium either in human corpuscles or in those of animals.

Contemporary and subsequent workers, however, challenge these statements, and Cowie and Calhoun⁽⁶⁶⁾, Jones and Nye⁽⁶⁷⁾, and Stanford and Wheatley⁽⁶⁸⁾ have actually estimated the amount of calcium present in the corpuscles. The figures recorded by these authors range from 1.4 to 8.7 mgs. calcium per 100 c.c. corpuscles. Although the available evidence regarding the mere presence or absence of calcium in the corpuscles is so conflicting, it seems unreasonable to suppose that the corpuscles are entirely devoid of calcium/

calcium, since they are bathed by a calcium-containing medium. Most of the negative evidence has been obtained by indirect methods, and without any appreciation of the modifications produced in the calcium content of the fluid portion of the blood by the processes of citration and coagulation.

Normal figures for whole blood are given by Lyman⁽⁶⁹⁾ as 6.1 mgs. per 100 c.c. for males and 7.1 mgs. for females; by Kramer and Tisdall⁽⁷⁰⁾ as 5.3 to 6.8; by Alport⁽⁷¹⁾ as 5.8; by Jones⁽⁷²⁾ as 8.8; and by Jones and Nye⁽⁶⁷⁾ as 9.4 (children).

Greenwald⁽⁷³⁾, using heparin as an anticoagulant, found the calcium content of the plasma to be identical with that of the serum, and Kramer and Tisdall⁽⁶³⁾ state that this is true also for citrated plasma.

Several experiments have been carried out by the author in order to ascertain if the recorded statements of other observers concerning the calcium content of plasma, citrated plasma, and serum, respectively, are to be relied upon.

Plasma was obtained in the following manner: Blood was withdrawn from a cat's carotid into a paraffined syringe, and a sample collected under paraffin in a centrifuge tube which had previously been placed in an ice mixture and thoroughly cooled. A second sample was allowed to clot, and to a third was added sodium citrate. The first sample was immediately/

immediately centrifuged for three minutes, and then a measured quantity of the supernatant serum was quickly withdrawn for calcium analysis. By this method unclotted and unadulterated plasma was obtained with comparative ease. The calcium content of the serum and citrated plasma of the same sample were also estimated.

The results obtained were as follows:-

Mgs. calcium per 100 c.c.		
True plasma	Plasma from citrated blood	Serum
12.1	10.0	10.0
11.6	10.4	10.3
11.1	10.05	10.0
12.15	9.5	10.10
11.05	9.15	9.0
12.7	11.50	11.30

It will be seen from these figures that the calcium content of the serum is considerably lower than that of the plasma, to the extent of about 10%, and that the plasma undergoes a reduction in its calcium content of a similar order to that of serum, as a result of citration.

The nature of the action of citrate on the plasma calcium was further investigated. Plasma was obtained in the manner described. A sample was analysed/

analysed for calcium unchanged and the remainder was treated with sodium citrate. A sample of this citrated plasma was shaken up and the calcium content estimated; the remainder was centrifuged for five minutes at high speed, and the upper layer of fluid analysed. The calcium content of the serum was also estimated.

The results obtained were as follows:-

Expt.	True plasma	Citrated plasma	Citrated & centri-fuged plasma	Serum
1	11.8	11.8	10.6	10.4
2	12.10	12.0	10.8	10.3
3	11.70	11.60	10.7	10.5
4	11.80	-	10.20	10.2
5	9.95	10.05	8.8	8.8
6	10.0	10.0	9.1	-

From these results it would appear that the addition of sodium citrate to plasma results in a precipitation of a certain proportion of the calcium present in the plasma. With the amounts usually added to prevent coagulation this reduction is small, so that the calcium content of the citrated plasma is very near to that of the serum, but since in one instance in the previous table the calcium content of the citrated plasma was decidedly lower than that of the serum, the amount of precipitation would seem to/

depend on the amount of citrate added. This reduction in the calcium content of the plasma is of course insufficient to account for the inhibiting influence of citrates on the process of coagulation of blood.

It is now generally recognised that on account, inter alia, of the variability of the corpuscle volume, figures obtained from an analysis of whole blood are of little value for purposes of comparison, and that the estimation of the serum calcium affords the most accurate data. It is not proposed to deal with the widely diverging figures found for the serum calcium before the introduction of satisfactory methods of analysis.

Kramer and Tisdall⁽⁶⁰⁾ record ten normal human sera containing 9.5-10.5 mgs. calcium per 100 c.c. with six between 9.5 and 10.0 mgs.; Kramer and Howland⁽⁷⁴⁾ found seven normals within a range of 9.3-9.9, while Watchorn⁽⁷⁵⁾ gives a higher figure for the normal, but here too, the range 10.0-10.8, is small.

The author has obtained the normal value for the serum calcium in ten normal healthy men; the estimations were done in duplicate, using Kramer and Tisdall's method, and gave results varying from 9.4 to 10 mgs. per 100 c.c. serum.

Normal/

Normal Subjects.

	<u>Mgs. calcium per 100 c.c.</u> <u>serum.</u>
I. _____	9.6
II. _____	9.7
III. _____	9.6
IV. _____	9.9
V. _____	9.5
VI. _____	9.6
VII. _____	9.5
VIII. _____	9.5
IX. _____	9.7
X. _____	10.0

Subsequent determinations of the serum calcium in these subjects at intervals of several months showed practically identical figures.

All investigators have agreed that only a portion of the calcium present in the serum is free. There are three possible forms in which calcium may occur in the serum, namely, as calcium ions, as undissociated molecules in equilibrium with the ions, and as a non-ionizable compound with some of the organic constituents of the serum. The sum total of the calcium present in the ionic state or as undissociated molecules may be determined by dialysis or by ultra-filtration. The method of compensation dialysis/

dialysis of serum gives variable results in the case of the inorganic constituents because of the existence of a Donnan membrane equilibrium on the sides of the membrane. The older work on ultra filtration, which yielded very variable results, was obviously fallacious on account of the high pressures employed for filtration since any labile compounds between the protein and inorganic constituent may have been decomposed by the excessive pressures.

Recent workers using the method of ultra filtration, either through collodion membranes or parchment, have employed much lower pressures, in most cases approximating to the average systolic blood pressure, and the results obtained have been much less variable. By this method Cushny⁽⁷⁶⁾ found 60-70% of the calcium present in ox serum to be filterable, and Neuhausen and Pincus⁽⁷⁷⁾ found 50-70% filterable calcium in pig serum. Since no regular precautions appear to have been taken by these workers in order to standardise the membranes used for filtration, the results obtained by them for the different animals are remarkably constant, especially as the two latter workers filtered very small amounts of serum.

The present author has carried out a series of experiments on collodion filtration of serum in order to ascertain the relative amounts of diffusible and non-diffusible calcium in this fluid. The collodion tubes were prepared as follows:

A collodion solution of the requisite viscosity was prepared by dissolving pure celloidin in a mixture of equal parts of methylated spirit and ether, in the proportion of 1 part celloidin to 10 of the ether spirit mixture. This was poured into test tubes of 4" x 1" dimensions; it was poured off immediately and the tubes allowed to drain for fifteen minutes. The thin film of collodion adhering to the inner surface of the tubes was withdrawn while the tubes were immersed in distilled water, and the thimble so obtained used as a filter by fixing a perforated rubber cork into the open end and connecting the cork to a pressure bottle. The pressure used for filtration was half an atmosphere.

The tubes were first of all subjected to air pressure till all obvious moisture had disappeared from the inside of the tube, and no more appeared on the outside after the excess which had been forced through had been removed by means of filter paper. Thereafter serum was put into the tubes and pressure again applied. When filtration had been in progress for fifteen minutes, the serum was removed from within the tubes, the outsides of which were dried with filter paper. In this way any water present in the interstices of the membrane was displaced, and dilution of the filtrate during the process of filtration was therefore avoided.

Filtration/

Filtration was allowed to progress for not longer than seven hours since if continued for a longer period, the filtrate contained a greater concentration of calcium, and no doubt this was due to a disturbance of the equilibrium between the different forms of combination of calcium in the serum.

The following table illustrates the influence of unknown quantities of moisture in the membrane, and of the time of filtration, on the calcium content of the filtrate:

<u>Time of Filtration</u>	<u>Mg. Ca/100 c.c. filtrate.</u>
---------------------------	--------------------------------------

(a) Membrane surface dried
by filter paper.

0	-	3	hours	—————	4.2 mgs.
3	-	7	"	—————	7.0 mgs.
7	-	22	"	—————	8.5 mgs.

(b) /

(b) Membrane treated by air pressure and preliminary filtration.

<u>Time of Filtration</u>	<u>Mg. Ca/100 c.c. filtrate</u>
0 - 3 hours _____	7.5
3 - 7 " _____	7.55
7 - 22 " _____	8.85

Whole serum 9.0 mgs. Ca/100 c.c.

The following results show that after being treated as described different collodion membranes filter equally.

Mg. Ca/100 c.c. whole serum used in filtration _____	10.8
Mg. Ca/100 c.c. filtrate: Filter 1 _____	7.3
Filter 2 _____	7.3
Filter 3 _____	7.25
Filter 4 _____	7.25

Using this method of ultra-filtration through collodion membranes the relative proportions of diffusible and non-diffusible calcium have been determined in normal human sera, and in the serum of the cat and the ox. The following results were obtained: /

Sample	Human		Ox		Cat	
	Mg.Ca/ 100cc. serum	% ^{as} Diff- usable calcium	Mg.Ca/ 100 cc calcium	% as diff- usable calcium	Mg.Ca/ 100 cc calcium	% as diff- usable calcium
1	10.20	72.8	10.05	66	10.85	67
2	9.8	65.5	9.8	64	10.80	57
3	10.2	66.0	10.0	64	11.80	61
4	10.0	60.0			10.80	67
5	10.0	73.0			10.40	57
6	9.6	72.0			10.70	67
7	9.8	70.0			10.0	66
8	10.4	71.0			9.80	61
9	10.2	62.0			10.30	63
10	10.1	64.0				

The diffusible portion of the serum calcium appears to be fairly constant in the species examined, and to amount to from 57 to 75 per cent. of the total, being somewhat lower in the cat and the ox than in man. That only a fraction of the diffusible calcium of the serum is in the form of calcium ions is shown by the work of Neuhausen and Marshall⁽⁷⁸⁾. These workers, using a calcium electrode method for the estimations, found only 2 mg. calcium per 100 c.c. serum to be present as ions, the mean error of the estimation being/

being $\pm 20\%$. Brinkman and Van Dam⁽⁷⁹⁾ have estimated the ionic calcium in serum ultra-filtrate by a method based on the solubility product $\text{Ca} \times \text{C}_2\text{O}_4$ and found approximately 2 mg. calcium ions per 100 c.c. serum.

Interesting papers dealing with the form in which calcium exists in the blood have recently been published by Holt, La Mer and Chown⁽⁸⁰⁾. These authors believe that the blood serum is normally supersaturated with $\text{Ca}_3(\text{PO}_4)_2$ to the extent of more than 200%. On shaking serum with solid calcium phosphate there is a reduction in the serum calcium from 10.0 to 1.8-2.5 mg. per 100 c.c., and in phosphate from 3.5 to 1.5-2.1 mg. with precipitation of tertiary calcium phosphate. No such reduction occurs on shaking without the solid phase. Hence the fluids bathing the bone matrix, where solid calcium phosphate is present, can readily give up their surplus and so enable calcification to take place. They explain the supersaturation as due to the sudden change during absorption from the acid reaction of the small intestine to the alkaline reaction of the tissues, a change which produces PO_4''' from $\text{H}_2\text{PO}_4'$ and HPO_4'' with consequent formation of $\text{Ca}_3(\text{PO}_4)_2$ at a rate much greater than it can be precipitated.

The Control of the Serum Calcium.

The calcium content of the serum is maintained at a remarkably constant level, and numerous observations of its amount in various physiological states and pathological conditions only serve to emphasise this fact. This being the case it is reasonable to assume either that calcium plays a part of no importance in the bodily economy, or that a very delicate mechanism exists whereby any tendency to disturbance of the normal calcium metabolism by outside influences is immediately rectified. Since, however, there are a few well known pathological conditions which are invariably accompanied by, and are now rightly considered to be due to, a disturbance of calcium metabolism, and since in several other conditions deranged calcium metabolism is occasionally evident, the latter alternative may be fairly accepted, and the disturbance of calcium metabolism considered as being due to improper functioning of the whole or part of this regulating mechanism.

Apart from variations in the supply and availability of exogenous calcium, which have already been discussed, there are three factors which are known to influence the serum calcium.

Firstly, vitamin D is necessary for the complete utilisation of calcium by the organism, and its indispensability is obvious when the disastrous effects/

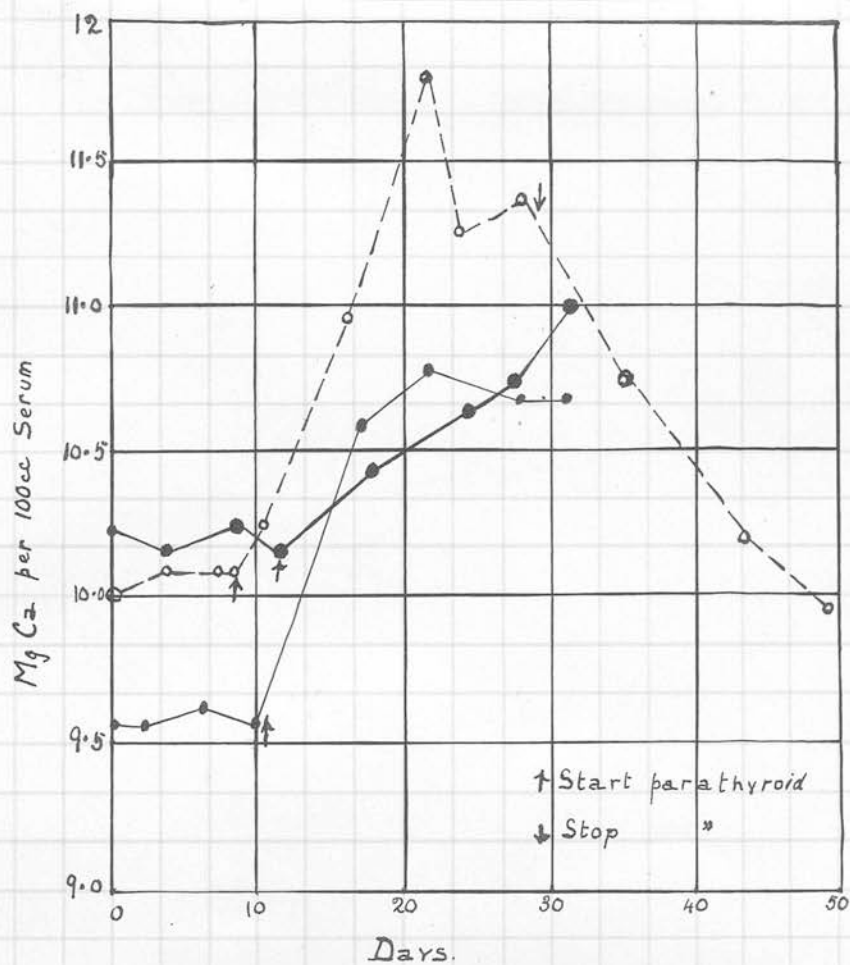
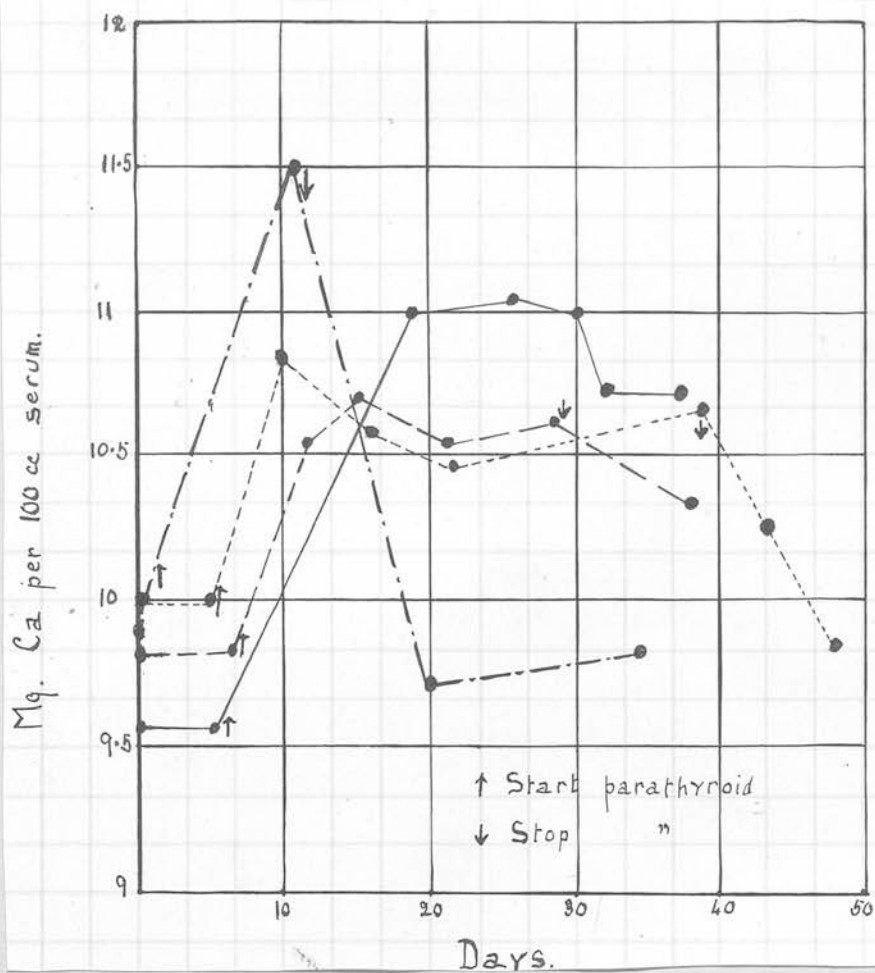
effects which follow its removal are observed. Whether this factor acts by increasing the absorption of calcium from the diet, or by facilitating its utilisation by the tissues is unknown, but when one considers the minute quantities which are required, and also that under the influence of ultra-violet rays the organism itself may produce its own vitamin D requirement, even when previously suffering from a vitamin D deficiency, the latter possibility appears to be the most likely. Experiments which claim to show that absorption of calcium is stimulated by vitamin D merely reveal the fact that after addition of this factor less calcium is excreted than before, and since it has been definitely shown in the preceding pages that the major part of the calcium absorbed is re-excreted via the large bowel, such experiments can only be interpreted as demonstrating a calcium retention, and throwing no light whatsoever on the process of calcium absorption.

Secondly, the parathyroid glands through the medium of their secretion play a very definite and important part in the mechanism controlling calcium metabolism. A great deal of evidence, both experimental and clinical, has been brought forward in support of this statement; much of it has been questioned, and it is only recently that definite proof has become available that the parathyroid glands secrete an active substance which regulates at least one/

one phase of this process. MacCallum and Voegtlin⁽⁸¹⁾ showed that administration of calcium salts decreased the symptoms in tetania parathyreopriva, and their observation has since been amply confirmed. Conversely MacCallum, Lambert and Vogel⁽⁸²⁾ obtained hyperexcitability of the nerves by perfusing blood which had been dialysed against calcium free fluid, but not when the blood had been dialysed against a fluid containing calcium.

In tetania parathyreopriva occurring in adults low serum calcium values have also been found by Kramer and Tisdall⁽⁶⁰⁾, and in a case observed by the present author the serum calcium was found to be reduced to 6.9 mgms. per 100 c.c. serum. There is thus ample evidence that removal of the parathyroid glands results in a lowering of the serum calcium, which is apparently responsible for the ensuing nervous hyperexcitability. The theory advanced by Noel Paton and his co-workers^(83,84,85,86,87) that guanidine or its derivatives accumulate in the body in the absence of the parathyroid glands and by a toxic action on the motor nerve endings produce tetany has been disproved by the experiments of MacCallum and Vogel⁽⁸⁸⁾, Greenwald⁽⁸⁹⁾, and Collip and Clark^(90,91) who were unable to demonstrate the presence of a tetany producing poison in the blood of parathyroid-ectomised animals.

Graph 2.



Berkeley and Beebe⁽⁹²⁾ in 1909 prepared an extract from beef parathyroids from which they separated a nucleoprotein that appeared to them to have active properties when administered to parathyroidectomised animals. Biedl⁽⁹³⁾ in 1916 transplanted the parathyroids of a dog to the spleen and then performed a thyroidectomy. This was not followed by tetany, but when the spleen was removed, months later, fatal tetany developed within twenty-four hours. Similarly Borchers⁽⁹⁴⁾, in 1919, stated that a single large parathyroid graft is adequate to obviate symptoms of post-operative tetany in the human subject.

The present author experimenting in 1925-25 with an extract of beef parathyroids prepared by Messrs Armour and Co. found that this preparation produced a very definite rise in the serum calcium in human subjects. The results are given in Graph 2.

Hanson^(95), Berman^(96), and Hjort, Robinson and Tendick^(97), have since published similar figures showing a rise in the serum calcium following the administration of extract of the parathyroid glands obtained by them by various methods of extraction.

It was not, however, until Collip^(98) in 1925 evolved a method of extraction of the parathyroid glands by means of acid digestion and isoelectric precipitation that a potent extract was obtained that could be relied upon to cause a definite rise in the serum calcium in every case. Collip found that this extract - (1) prevented tetany from occurring after parathyroidectomy.

(2) abolished tetany already present following parathyroidectomy.

(3) that the discontinuance of its use after parathyroidectomy was followed by tetany.

(4) raised the serum calcium.

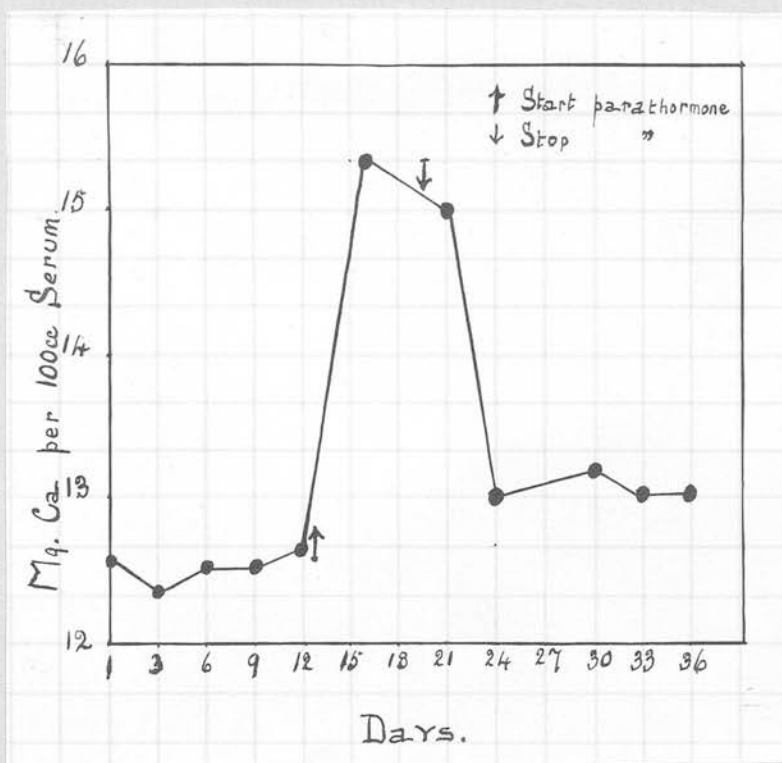
(5) was active orally, hypodermically or intravenously.

Since the rise in the serum calcium produced by a single dose of the extract was proportioned to the amount given, he was able to standardise ~~physiologically~~ physiologically the preparation. A provisional unit has been

arbitrarily defined (Collip⁽⁹⁹⁾) as one-hundredth of the amount of the extract that will produce an average increase of 5 mgms. in blood serum calcium in a normal dog of 20 kgm. weight over a period of 15 hours.

(98)
It was further noted that repeated injections of even small amounts of the extract at intervals of

Graph 3.



From Day 15 to 18 the patient suffered from headache, giddiness, weakness, nausea and vomiting.

a few hours caused in dogs a much greater hypercalcemia (23 mgms. calcium per cent.) than a single dose. The increase due to each injection is superimposed upon the high level of serum calcium due to the previous injection. This hypercalcemia is dangerous and is accompanied by symptoms of anorexia, vomiting, diarrhoea, failing circulation, bradycardia, coma and death. Apart from the great increase in the serum calcium no other unequivocal changes in the blood chemistry have been noted. Looney⁽¹⁰⁰⁾ found a hypercalcemia in human subjects after successive doses of Collip's parathyroid extract. The present author also produced symptoms of headache, nausea, vomiting and giddiness in a patient suffering from "fragilitas osseum" as a result of administration of Collip's parathyroid and there was a coincident high serum calcium value. The serum calcium figures are recorded in Graph 3. It will be noted that in this condition the initial serum calcium value was very high, and this is probably a feature of the disease.

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Crile⁽¹⁰¹⁾, Snell⁽¹⁰²⁾, Lissner and Shepardson⁽¹⁰³⁾
and Collip⁽¹⁰⁴⁾ have found that the administration of
Collip's extract to human subjects suffering from
tetania parathyreopriva caused a rise in the serum
calcium and cured the condition.

It would appear then that the parathyroid glands
produce an active principle which controls the level of
the serum calcium. In so doing these glands resemble
known internally secreting glands of the body, and may
therefore be classed along with them. The active
principle evolved by the parathyroid glands seems to
be of the nature of a hormone, both in the absence of
which and in its presence in excess, a definite train
of symptoms develops.

The means whereby the parathyroid hormone exerts
its controlling action on the serum calcium remain to
be examined. Clearly the hormone may affect the
absorption or excretion of calcium, or may exert some
controlling action on the equilibria between the
different forms of combination in which calcium exists
in the blood and between the concentration in the
blood and in the tissues. In the work recorded below
the author has aimed at obtaining some direct evidence
as to the mode in which the parathyroid hormone exerts
its action.

Since/

Since many of the projected experiments necessitated the frequent drawing of blood samples and consequently involved considerable haemorrhage, it seemed advisable to test first the effect of haemorrhage itself on the calcium content of the serum.

Cats were used throughout as the experimental animals, and although these were bled freely at intervals of half an hour, to the extent of a sixty per cent. diminution of the estimated total blood volume, and a correspondingly large loss of haemoglobin, there was no reduction in the serum calcium even at death. The results of these experiments are given in Table II.

Table II /

Table II.

The effect of haemorrhage on the serum calcium in the cat.

No.	Wt. of cat. in grams	Time in mins.	% Hae-moglo-bin.	Corp. vol. hae-mato-crit.	Mg. Ca per 100cc. serum	Total hae-morr-hage cc.	% re-duction in serum Ca.	% reduction in haemo-globin.
1	2800	0	66	35	9.90	-	-	-
		30	55	32	9.84	-	-	-
		60	53	29	9.87	-	-	-
		90	50	24	9.90	47	Nil	15
2	3000	0	65	31	10.3	-	-	-
		40	-	-	10.25	-	-	-
		80	-	-	10.3	-	-	-
		120	50	25	10.3	-	-	-
		150	-	-	10.4	-	-	-
		180	40	21	10.3	81	Nil	37.4
3	3000	0	70	-	9.5	-	-	-
		60	-	-	9.5	-	-	-
		120	-	-	9.5	-	-	-
		180	47	-	9.5	57	Nil	33
4	2980	0	66	44	9.90	-	-	-
		40	-	-	9.90	-	-	-
		80	-	-	9.90	-	-	-
		120	-	-	9.90	-	-	-
		150	50	30	9.90	80	Nil	24
5	3000	0	67	-	11.0	-	-	-
		60	-	-	10.95	-	-	-
		120	-	-	10.95	-	-	-
		180	40	-	11.0	90	Nil	40
6	3020	0	-	-	9.25	-	-	-
		60	-	-	9.25	-	-	-
		120	-	-	9.25	-	-	-
		180	-	-	9.25	89	Nil	-

Table III. /

The Effect of Parathyroid Hormone on the Blood
Serum of Cats.

The parathyroid hormone used in the earlier experiments was kindly presented to the author by Dr McNee, who had obtained it from Dr Collip; later the commercial preparation "Parathormone Lilly", extracted by Collip's method was employed. Table III. shows the effect of subcutaneous injections of these preparations on cats.

Table III.

Effect of Collip's parathyroid extract given subcutaneously, on the serum calcium in cats. (0.5 c.c. (10 units) parathyroid extract given after withdrawal of first blood sample).

Expt.	Wt. of cat in grams.	Mg. Ca per 100 c.c. serum		Increase
		Before injection	15 hrs. after injection.	
1	2110	10.2	12.4	2.2
2	2440	9.7	11.9	2.2
3	2800	9.8	12.8	3.0
4	3360	11.0	14.2	3.2
5	2800	10.4	14.1	3.7

In every case it will be noted the serum calcium rose to an extent which, though less than would, presumably, have been observed in dogs, was well beyond the limits of experimental error and was undoubtedly real. The effect of intravenous injection of the hormone was next investigated. The cat was anaesthetised/

anaesthetised by means of paraldehyde and ether and a sample of blood having been withdrawn from the carotid, parathyroid hormone was given intravenously and thereafter blood was withdrawn at frequent intervals.

Table IV.

(a) Effect of intravenous injection of "Parathormone" in cats anaesthetised with paraldehyde and ether.

<u>Expt.</u>	Wt. of cat in grams.	Mg. Ca per 100 c.c. serum before injection.	Parathormone injected. c.c.	Mg. Ca per 100 c.c. serum.		
				30 min.	120 min.	160 min.
1	2210	10.1	0.5	10.9	11.8	11.1
2	2900	10.0	0.5	11.6	12.0	10.4
3	3300	10.4	0.5	10.9	12.0	11.6

(b) Effect of intravenous injection of "Parathormone" in decerebrate and pithed cats.

<u>Expt.</u>	Wt. of cat in grams	Mg. Ca per 100 c.c. serum before injection.	Parathormone injected c.c.	Mg. Ca per 100 c.c.	
				45 mins.	90 mins.
1	3400	10.30	0.6	10.70	11.65
2	2890	9.6	0.6	11.70	12.00

The results of these experiments (Table IV (a)) show that under these conditions the hormone exerts its action much more rapidly than when given hypodermically, the maximum rise in the serum calcium being attained in about two hours. The magnitude of the rise, however, is not greatly increased.

In/

In Table IV (b) are shown the results of experiments in which the parathyroid hormone was given intravenously to cats after decerebration which included removal of the pituitary gland, the spinal cord having also been destroyed. The rise in the serum calcium was of the same magnitude as that occurring in anaesthetised animals. It is evident therefore that the effect of the parathyroid hormone is not exerted through the central nervous system.

It may be remarked at this point that exposure to air rapidly inactivated the hormone (Table V).

Table V.

Effect of subcutaneous injection of Collip's parathyroid extract, which had been exposed to air and kept at 37° C. for 24 hours, on the serum calcium in cats.

Expt.	Wt. of cat in grams	Mg.Ca/100 c.c. serum before injection.	Parathyroid extract injected c.c.	Mg.Ca per 100 c.c. serum 15 hours.
1	1980	9.5	0.5	9.5
2	2350	10.5	0.5	10.5

The/

The Effect of Parathormone on the Absorption
of Calcium.

The direct measurement of the absorption of calcium is beset with many difficulties. Not only is the faecal output a combination of unabsorbed residues and of re-excreted calcium, the relative amounts of each being unknown, but the amount retained - which is all that can be measured - is obtained by difference from estimations of the total intake and total output, and is never more than a small fraction of either. The margin of error in such experiments is therefore considerable. Nor is it desirable to take the urinary excretion as an index of the amount of calcium absorption since it varies very considerably from day to day even under apparently standard conditions.

Briefly, the method used by the author consists in the complete removal of the alimentary canal distal to the oesophagus, i.e. the whole of that portion from which absorption may conceivably take place. If, under these conditions, the parathyroid hormone is able to exert its full effect on the serum calcium, it must mobilise calcium from some internal source or, possibly, act by controlling the rate of excretion. On the other hand, its inability to raise the serum calcium would indicate that normally the hormone draws on an external supply.

The/

The technique of the experiments was as follows: Paraldehyde and ether were used to produce anaesthesia. The abdomen having been opened, the duodeno-jejunal junction was identified and the gut severed between two ligatures at this point. The rectum was then drawn up out of the pelvis and cut across between ligatures close to the anus. The superior and inferior mesenteric arteries were ligated and the small and large intestine removed entirely. The duodenum was separated from the head of the pancreas, branches of the pancreatico-duodenal arteries being ligated. A series of ligatures were tied along the lesser and greater curvatures of the stomach from the pylorus to the cardia including the gastric vessels in the lesser omentum, and in the anterior layer of the greater omentum. The lower end of the oesophagus was ligated and cut across. The pylorus and first part of the duodenum were carefully separated from the portal vein, superior pancreatico-duodenal, splenic, and hepatic arteries, and the stomach and duodenum were removed by severing the omenta along the curvatures of the stomach. In this way the alimentary canal distal to the oesophagus was dispensed with, leaving the liver, spleen, and pancreas in situ, receiving an almost intact blood supply, that portion only of the portal circulation arising in the gut having been interfered with. The integrity of the arterial supply and venous/

venous return to these organs was in every case proved by the presence of arterial pulsation and by inspection of the venous flow. Where exclusion of liver, spleen and pancreas from the circulation was desired, the procedure was similar to that employed for evisceration alone, but it was unnecessary to separate the duodenum from the head of the pancreas. The hepatic artery and portal vein were ligated and cut in the free border of the lesser omentum, and the spleen and pancreas were removed entirely, after ligation of their respective arteries and veins.

At intervals blood was withdrawn through a cannula in the carotid artery, and injections were made through a cannula inserted in the external jugular vein. The animals were kept on artificial respiration and despite the extensive surgical interference, their condition remained satisfactory throughout the experimental period. It was necessary, as a preliminary, to the actual experiments, to ensure that in them the only variable condition was the presence or absence of added parathyroid hormone, since there were two other factors which might conceivably have caused an alteration in the calcium content of the blood. Firstly it had to be shown that the operative shock was without effect in this direction. Secondly, in removing the whole of the alimentary canal, not only had absorption of calcium been prevented, but one of the possible excretory routes had also been cut off. Control/

Control experiments in which evisceration was performed but no parathyroid hormone given showed that over a period of three hours, the serum calcium remained absolutely unchanged. Whether the two factors are individually without effect, or whether their effects cancel one another was not determined, but was, in this case, a matter of no importance.

The results of a number of experiments on the effect of parathyroid hormone on the serum calcium of eviscerated cats are given in Table VI.

Table VI.

The effect of evisceration and the removal of the liver, spleen and pancreas on the serum calcium in cats.

Expt.	Wt. of cat in grams	Mg. Ca per 100 c.c. serum before removal.		Mg. Ca per 100 c.c. serum.	
				45 mins.	90 mins.
1	2860	9.80	Intestine, liver, spleen & pancreas removed	9.80	9.80
2	3950	10.50		10.45	10.45
3	3800	9.90		9.85	9.90

Table VII /

Table VII.

The effect of intravenous injection of parathormone on the serum calcium of eviscerated cats.

Expt.	Wt. of cat in grams	Mg. Ca per 100 cc. serum before injection	Parathormone injected (c.c.)	Mg. Ca per 100 c.c. serum.	
				45 mins.	90 mins.
1	2760	9.0	0.7	9.8	11.2
2	2600	9.4	0.7	11.2	11.8
3	3500	8.4	0.7	11.4	10.2
4	2990	9.6	0.7	10.6	10.8
5	3330	10.0	0.7	11.6	11.2
6	3290	8.9	0.7	10.0	10.8

Table VIII.

The effect of parathormone on the serum calcium of cats in which the liver, spleen, pancreas, intestine and stomach have been removed.

Expt.	Wt. of cat in grams	Mg. Ca per 100 cc. serum before injection	Parathormone injected (c.c.)	Mg. Ca per 100 c.c. serum.	
				45 mins.	90 mins.
1	4050	9.80	0.6	11.00	11.35
2	2980	10.30	0.6	10.70	11.65
3	4010	9.90	0.6	10.60	11.10

It will be noted that in every case the hormone produced a rise in the serum calcium, and comparison of these results with those given in Table IV shows that/

that the magnitude of the rise is as great in eviscerated as in normal animals. It seems fair, then, to conclude that the parathyroid hormone exerts its influence on the calcium content of the blood without drawing on external sources of calcium, i.e. without stimulating calcium absorption. Further, the liver, spleen and pancreas, have no special function as internal sources of calcium (though of course they may be used as reserves in the same way as other tissues) nor do they appear to influence the action of the hormone in any way.

The effect of the parathyroid hormone on the excretion of calcium by the large bowel was investigated using a method similar to that employed by the author and already described, in the study of calcium excretion. An injection of the hormone was given at the end of a three hour control period. That the hormone was active was shown in each case by the withdrawal of blood samples at intervals and estimation of the serum calcium. The results are set forth in Table IX, and show the hormone to have little or no effect on the intestinal excretion of calcium.

Table IX /

Table IX.

The effect of intravenous injection of parathormone on the excretion of calcium by the large intestine in cats.

<u>Expt.</u>	Wt. of cat in grams	Mg. Ca in large intestine after 1st 3 hr period	Mg. Ca per 100 cc. serum in injection.	Parathormone injected. cc.	Mg. Ca in large intestine after 2nd 3 hr. period.	Mg. Ca per 100 cc. serum 3 hrs after injection.
1	2790	0.74	10.6	2	2.0	12.0
2	2400	0.70	9.9	2	0.72	11.5
3	2300	0.64	9.8	2	0.80	11.7
4	3050	0.60	10.0	2	0.60	11.8

In only one experiment was a definite increase obtained, but on the other hand, in no case was there a decrease. The latter, as has been pointed out, is the important finding, and indicates clearly that the parathyroid hormone does not control the rate of excretion of calcium by way of the large intestine. The fact that little or no increase in the calcium excretion was observed in these experiments is not surprising in view of the small rise in the serum calcium produced by the parathyroid hormone compared with that following the injection of calcium salts.

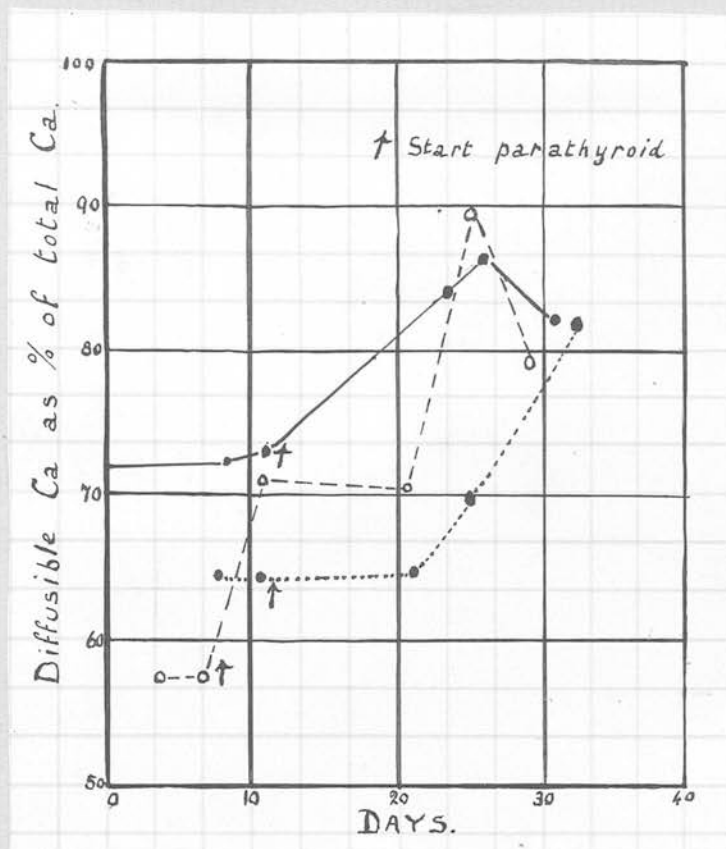
Indeed when one considers the amount of calcium excreted during the control period, it seems hardly possible that the parathyroid hormone could produce the observed rise in the serum calcium by diminishing this/

this amount. The average of a number of experiments shows that the total excretion of calcium in the control period of three hours is 0.8 mg., the greatest excretion observed being just over 1.0 mg. Now in all my experiments the serum calcium has been at least 1.0 mg. per 100 c.c. higher at the end of the three-hour period than at the beginning, and the net rise has usually been greater than this. The smallest cat used in these experiments weighed 2500 grams, which means an approximate blood volume of 200 c.c. and therefore at least 100 c.c. of serum. Hence an increase of 1 mg. per 100 c.c. in the serum calcium involves the mobilisation of 1.0 mg. calcium - at least - an amount greater than the total excretion during the period in which this rise has taken place. Obviously then, control of the rate of excretion cannot be the main mode of action of the parathyroid hormone. When one adds the experimental finding that the intestinal excretion, which accounts for much more than half of the total, is not diminished at all, it follows that the hormone does not act by controlling the rate of calcium excretion.

Alteration/



Graph 4.



Alteration in the physical state of the serum calcium following the administration of parathyroid extract.

It is reasonable to suppose that the calcium mobilised by the parathyroid hormone is readily diffusible, and that, therefore, the administration of the hormone will be followed by a rise in the ratio of diffusible to total calcium in the serum. To test this hypothesis Armour's parathyroid extract was administered by the mouth to three human subjects and the diffusible portion of the serum calcium estimated by means of collodion filtration.

The results given in Graph 4 show a marked rise in the percentage of diffusible calcium.

Several workers have shown that parathyroidectomy produces a relatively greater fall in the diffusible than in the non-diffusible calcium (Salveson and Linder⁽¹⁰⁵⁾, Trendelenberg⁽¹⁰⁶⁾, Moritz^()).

Hence it seems that on the amount of parathyroid hormone depends primarily the concentration of the readily diffusible calcium. This conclusion is to some extent in agreement with the suggestion of Greenwald and Gross⁽¹⁰⁷⁾ that the parathyroid hormone is, or is necessary for the production of a substance (which Greenwald considers to resemble citric acid) capable of retaining in solution the excess $\text{Ca}_3(\text{PO}_4)_2$ which/

which Holt, La Mer and Chown⁽⁸⁰⁾ have shown to be present in blood.

The third factor which may control the serum calcium level is the neutrality regulating mechanism of the body. Variations in the alkali reserve are accompanied by definite alterations in the amount of calcium present in the serum. The most convincing example of such an alteration is afforded by the work of Stewart and Haldane⁽¹⁰⁸⁾ who produced a marked decrease in the serum calcium following the oral administration of sodium bicarbonate in large doses, and an equally marked rise resulting from the ingestion of ammonium chloride, and the inhalation of carbon dioxide. Thus any tendency to an alteration of the P_H of the blood and body fluids towards the alkaline side would appear to be accompanied by a lowering of the serum calcium, while a reduction in the alkali reserve and a state of acidosis is associated with a rise in the serum calcium.

Whether these variations form a part of the mechanism whereby the P_H of the body is maintained at a constant value, or whether they are merely the result of the activity of this mechanism and take no part in its operation cannot at present be definitely stated.

Rona and Takahashi⁽¹⁰⁹⁾, Paasen⁽¹¹⁰⁾ and Brinkman⁽¹¹¹⁾ hold that the concentration of calcium ions depends on the P_H and the bicarbonate reserve, but/

but Meysenberg and McCann⁽¹¹²⁾ were unable to demonstrate any alteration in the amount of diffusible calcium as a result of varying the CO₂ content of the serum.

The present author has observed the effect of the intravenous injection of sodium bicarbonate on the serum calcium in cats. This procedure was found to be followed by a rapid and marked fall in the serum calcium level. This fall in the serum calcium could be prevented if parathyroid hormone was injected immediately after the sodium bicarbonate, but it is impossible to say whether this mutual antagonism is due to an action on the same tissues. That it does not result from the parathyroid hormone producing an acidosis may be concluded from the recent work of Cantarow, Caven and Gordon⁽¹⁴¹⁾ who found that parathormone was without effect on the CO₂ combining power of the blood.

Table X.

(a) The effect of the intravenous injection of sodium bicarbonate on the serum calcium in cats.

Expt.	Wt. of cat in grams	Mg. Ca per 100 c.c. serum before injection	NaHCO ₃ injected gms.	Mg. Ca per 100 c.c. serum		
				30 min.	75 min.	100 min.
1	2110	10.8	0.8	10.0	9.4	8.8
2	2750	8.9	1.0	7.4	7.4	7.5

(b) /

Table X contd.

(b) The effect of simultaneous intravenous injection of NaHCO_3 and parathormone on the serum calcium in cats.

<u>Expt.</u>	Wt. of cat in grams.	Mg. Ca per 100 c.c. serum Before injection.	Parathormone(cc) NaHCO_3 (gms)	Mg. Ca per 100 c.c. serum		
				30 min.	75 min.	100 min.
1	2750	8.8	0.7 cc. 1.0 gms.	8.75	8.85	8.8
2	1870	10.2	0.7 cc. 1.0 gms.	10.0	10.2	10.0

In conclusion one may briefly summarise the results and conclusions in this thesis which are the outcome of the author's own experimental work.

(1) Calcium is absorbed from the alimentary tract much more readily when given in the form of calcium chloride than when given as the lactate.

(2) Calcium is excreted by the epithelium of the large intestine; this tissue forms the main excretory route for calcium.

(3) /

(4) The diffusible portion of the serum calcium amounts to from 60 to 70 per cent. of the total serum calcium in man, and from 50 to 60 per cent. of the total in the cat and the ox.

(5) The calcium content of the plasma is 10-20 per cent. greater than the calcium content of the serum.

(6) The addition of citrate to plasma causes a precipitation of 10-20 per cent. of the contained calcium.

(7) Citration of plasma or serum results in practically the whole of the contained calcium becoming diffusible.

(8) Extracts of beef parathyroid glands when administered orally, subcutaneously or intravenously, produce an increase in the serum calcium in man and in cats, and the diffusible portion is increased relatively to the total.

(9) If the increase in the serum calcium is very marked, symptoms of nausea, vomiting, weakness and giddiness may make their appearance. These subside on the withholding of the extract, and coincidently with the fall in the serum calcium.

(10) Parathyroid extract does not produce a rise in the serum calcium by stimulating the absorption of calcium from the digestive tract.

(11)/

(11) Parathyroid extract produces its effect independently of the central nervous system, pituitary gland, liver, spleen and pancreas. These structures are neither necessary to its action, nor do they act as stores of calcium.

(12) Parathyroid extract does not lessen the excretion of calcium, but probably increases it.

(13) Repeated and severe haemorrhage does not alter the serum calcium level in the cat.

(14) The intravenous injection of sodium bicarbonate produces a fall in the serum calcium. This fall does not occur if parathyroid extract is injected simultaneously with sodium bicarbonate.

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